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From: Arthur, Lisa
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Please provide the following references for 08/484,786:

Kajimura et al. J. CELL BIOL. 95, page 217A (Nov. 1982)

Larhammer et al. PNAS 79 3687-3691 (June 1982)

Long et al. EXPERIMENTIA 38, page 744- (1982)

Long et al. PNAS 79 7465-7469 (dec 1982).

Ploegh et al. PNAS 77 page 6081-6085 (Oct. 1980)

Owerbach et al. PNAS 80 3758-3761 (jUNE 1983).

THANKS

10035 Entire Amino Acid Sequence of HLA-DR β Chain Deduced From the Cloned of cDNA Sequence. Y. Kajimura, H. Toyoda, M. Sato, Y. Ike, S. Miyakoshi, J. Shively, S. Silver, S. Kaplan, S. Suggs, J. Huang, L. Ian, and K. Itakura. Department of Molecular Genetics, City of Hope Research Institute, Duarte, CA. 91010.

The HLA-DR antigens are composed of two noncovalently associated membrane glycoproteins of 35,000 daltons (α chain) and 29,000 daltons (β-chain). This class of antigen has a restricted cellular distribution, being present mainly on B cells and macrophages. They regulate various immune reactions and cell-cell interactions. It is of interest to characterize the structure of HLA-DR antigens.

Very recently we developed a screening method for the identification of any desired cloned DNA sequence. The general approach is to chemically synthesize a mixture of oligonucleotides which represent all possible codon combinations for a small part of the amino acid sequence of a given protein. Under stringent hybridization conditions only perfectly matched duplexes will form, allowing use of the mixture of oligonucleotides as a specific hybridization probe. This method was successfully applied to identify the cDNA clone for β-2 microglobulin and H-$2K^b$ antigen. We have constructed a cDNA library from a human lymphoblastoid cell line LG-2 (homozygous cell line in DR-1). The cDNA library is screened by synthetic oligonucleotide probes and two cDNA clones for DR-α chain were identified. These clones are identical, determined by restriction enzyme analysis and one of them is completely sequenced. We will report the detail structure of HLA-DR α chain.

10036 DNase I hypersensitivity of the 5' end of the Chinese hamster dihydrofolate reductase gene. Anjikhon, J.C.* and Hamlin, J.L.* Dept. of Biochem., Univ. of Virginia Sch. of Med., Charlottesville, VA. (Intr. by W.B. Looney).

Since the observation by Weintraub and Groudine (Science 193: 848-856, 1976) that transcriptionally-active chromatin is preferentially digested by pancreatic deoxyribonuclease I (DNase I), much effort has been directed at determining the precise location of the DNase I-hypersensitive sites within genes. We have examined the DNase I-sensitivity of the dihydrofolate reductase (DHFR) gene in a Chinese hamster ovary cell line which is resistant to methotrexate at a concentration of 400 μg/ml. These cells have approximately 1,000 copies of the DHFR gene, and we have cloned their genome in cosmids. The recombinant cosmids containing DHFR sequences have been mapped with restriction enzymes and subcloned in plasmids for use as probes on restricted genomic DNA after DNase I treatment of nuclei. When nuclei are treated with DNase I at concentrations of 0.5 to 10 units/ml for 3 min at 25° (which renders less than 0.2% of the DNA acid-soluble), a change in the restriction pattern of the DHFR gene is observed when digests are subsequently probed with 5'-genomic fragments. By restricting the DNA with different enzymes after DNase I treatment of nuclei, we have mapped the specific DNase I cleavage sites and the degree of sensitivity. We observed three equally sensitive sites in the 800 bp region lying 5' to the first exon and two sites within the 1 kb region spanning the first two exons. There are several other sites in the 5' region which are slightly less sensitive. When naked genomic DNA or a plasmid clone containing the 5' region of the DHFR gene are incubated with DNase I, there is no selectivity in the pattern of degradation. Thus, the DHFR gene, like many other eukaryotic genes, has a complex pattern of DNase I hypersensitivity in the region of the gene which is expected to contain regulatory information. Since DHFR is cell cycle regulated, certain features of the structure of chromatin in this region may prove to be related to periodic gene expression.

10037 The cDNA sequences of a human epidermal keratin and an actin: differential evolution of cytoskeletal proteins. I. Hanukoglu*, N. Tanese* and E. Fuchs, Department of Biochemistry, The University of Chicago, Chicago, IL 60637.

The cytoskeleton of most mammalian cells is comprised of microtubules, intermediate filaments, and microfilaments. Although, these structures are each assembled from one or a few polypeptide subunits, the proteins that are involved in the formation of intermediate filaments constitute the largest and the most diverse group of subunits. Nevertheless, all intermediate filaments have a similar fine ultrastructure. To explore the differential evolution and expression of the genes for these proteins, the cloned cDNAs that represent copies of mRNAs for keratins, actin and tubulin were identified within a library of cloned hybrid plasmids constructed previously in this laboratory. By positive hybrid-selection and translation, the keratin mRNAs were observed to fall into two classes: one class that codes for 56-58 kd keratins and the other for 46-50 kd keratins (Fuchs et al., Cell 27:75, 1981). In the present study we determined the DNA sequence of a cloned cDNA insert that is complementary to > 95% of the coding region for the 50 kd keratin. A comparison of the predicted amino acid sequence of this keratin with partial sequences of other intermediate filament proteins indicates that keratins may be the most distantly related members of this class of fibrous proteins. In contrast, the variation in the sequences of different forms of actin are minor. Our determination of the DNA sequence of a cloned cDNA insert complementary to a human cytoplasmic actin indicates that whereas the cytoskeletal actins are similar to the muscle actins, they show even less divergence across species. The finding that intermediate filament proteins can assume a much wider flexibility in their sequence than the actins or tubulins may indicate that these proteins have evolved to meet subtly different requirements in the cytoskeletal architecture of different cell types.

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10038 An Alternative View of Mammalian Repetitive DNA Sequence Organization. R.K. Moyzis*, J. Bonnet*, B.D. Crawford*, M. Dani*, P.J. Jackson*, J.R. Wu, and P.O.P. Ts'o. Division of Biophysics, The Johns Hopkins University, Baltimore, MD 21205, and Genetics Group, Los Alamos Scientific Laboratory, Los Alamos, NM 87545.

Recently, our laboratory presented biochemical and biophysical studies of the arrangement of repetitive DNA sequences in the Syrian hamster genome (Moyzis et al., 1981, J. Mol. Biol. 153, 841-870 and 871-896). These experiments suggested that a) traditional methods of measuring repetitive DNA sequence spacing potentially overestimate the amount of spacing distances shorter than 1 kb (kilobase), b) hyperchromicity experiments potentially underestimate the weight average length of repetitive DNA regions, and c) short 0.3 kb S1 nuclease resistant repetitive DNA duplexes are often produced from the reassociation of larger repetitive DNA regions. An alternative model of repetitive DNA sequence organization was proposed in which repetitive sequences are either frequently spaced at distances of 7 ± 2 kb or randomly spaced on a number average basis. The model further proposed that short 0.3 kb repetitive DNA sequences are often found in larger repetitive sequence clusters. These studies have now been extended to include the rat and human genomes, and similar results have been obtained. We have constructed "libraries" of rat, Syrian hamster and human genomic DNA in bacteriophage λ Charon 4A, as well as "libraries" of human S1 nuclease-resistant reassociated repetitive DNA in plasmid pBR 322 and bacteriophage M13mp7. Studies utilizing these libraries have confirmed our previous findings and have, in addition, indicated that the repetitive sequences present in long (>2 kb) S1 nuclease resistant structures are widely interspersed in the genome (at least once every 15-20 kb). This suggests that interspersed repetitive sequences are often longer than 0.3 kb, or that some members of 0.3 kb interspersed repetitive sequences are also found in longer repetitive sequence clusters. Repetitive sequences highly conserved between Syrian hamster and human DNA (approximately 10% of the total) are included in this interspersed long S1 nuclease resistant class.